REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons which follow.

I. Status of the claims

Claims 78-87 are pending. Applicants believe the Examiner has made a typographical error under the "Status of Claims" subheader at page 2 of the Office Action in referring to claims "78-88."

Claims 78, 81, 83, 85, and 86 have been amended. Claims 83 and 86 have been amended to replace the word "affects" with "modulates" to ensure that the preamble is consistent with the section (b) of those claims. Claims 78, 81, and 85 have been amended for the reasons that follow below.

Applicants also have amended the Specification to correct the grammatical errors identified by the Examiner.

II. The present claims do not lack utility because the specification clearly teaches that MCK-10 can be used in various assays to identify modulators, *i.e.*, antagonists or agonists, of MCK-10 activity

The Examiner states that "the [claimed] methods do not have any specific and substantial utility, or a well established utility, as determined according to the current Utility Examination Guidelines" under 35 U.S.C. § 101. Office Action at page 3. The Examiner further surmises that "the specification asserts that identification of agonists and antagonists of MCK-10 receptor is desirable, because such compounds could be useful in treating diseases such as cancer." She alleges, however, that the specification "has not established a substantial correlation between expression of the protein and cancer." The Examiner then goes on to relate that the level of gene expression in a cancer cell does not predictably correlate with an abundance of protein in cancer cells. Therefore, according to the Examiner, determining whether MCK-10 protein is involved in cancer "would require significant further research."

While the Examiner admits that "it is possible that after further characterization, this protein might be found to be directly involved in abnormal cell proliferation, and that modulation of the receptor would be therapeutic," she estimates that Applicants' claimed invention is "incomplete" because they allegedly have not performed such a characterization. Office Action at page 5.

For the reasons that follow below, Applicants regard the Examiner's interpretation of the presently claimed invention as incorrect. The Examiner's rationale for rejecting the claims lies in her belief that (1) Applicants did not prove that there is an overabundance of MCK-10 proteins in cancer cells; (2) one cannot correlate protein levels from mRNA transcript levels and, therefore, (3) it is unknown whether MCK-10 protein is overly-abundant in cancer cells. According to the Examiner, therefore, the present invention has no utility, because (4) there is no "nexus" between cancer and the molecules identified by the present invention.

Applicants reply by pointing out that the present claims are not directed to a method of using the abundance of MCK-10 protein in a cell as a diagnostic indicator for cancer. The present claims are directed to methods for identifying modulators of MCK-10 activity, *regardless* of how much MCK-10 protein is present in a cell. Accordingly, the Examiner's contention, that "even if the gene is expressed in many cancer cell lines and tumors, that does not necessarily mean that the protein is also expressed," has absolutely no bearing on the utility of the presently claimed invention.

The pending claims are directed to methods for identifying (i) an antagonist of MCK-10, (ii) a peptide that binds to MCK-10, and (iii) a compound that modulates MCK-10 activity. Thus, one utility of the presently claimed MCK-10 is for identifying molecules that modulate MCK-10 activity, because by doing so, it would be possible to apply the identified molecule and disrupt the cellular cascade that typically follows kinase activation. Hence, the identified molecules may be used modulate MCK-10 activity in cancer cells to alter cell proliferation signal pathways. However, the present claims are not directed to methods for identifying an abnormal cell type to which an identified modulator can be administered for treating that abnormality.

Clearly, MCK-10 can be specifically used to identify antagonists and agonists of MCK-10 activity and, therefore, the pending claims have a specific and substantial asserted utility. The skilled artisan would not reasonably doubt the credibility of Applicants' claim for utility, *i.e.*, that MCK-10 could be used for such purposes. Indeed, after learning that MCK-10 transcripts are overexpressed in cancer cells, the skilled artisan would understand that there exists *some* level of MCK-10 protein in cancer cells, the activity of which would likely induce a variety cellular responses. The *absolute level* or "threshold" amount of MCK-10 protein present in those cancer cells, however, is irrelevant to the analysis of the present claims.

Furthermore, there are numerous, post-filing date publications that corroborate Applicants' findings and assertions of utility. The skilled artisan would know, for instance, that "MCK-10" has received a number of alternative designations from those working in this field. Hence Vogel, *Faseb J.*, vol. 13 (suppl.), S77-S82, 1999, reported that the "discoidin receptor tyrosine kinase" *i.e.*, "DDR1," was "previously called NEP, Cak, Ptk-3, TrkE, **MCK-10**, RTK6, EDDR1, or NTRK4" (emphasis added; see the top paragraph in the right-hand column at page S77 of Vogel, 1999, which is appended to this paper). Thus, the skilled artisan would readily recognize that data, results, and theories stemming from experiments involving "DDR1" are just as applicable to Applicants' MCK-10 protein.

Accordingly, Vogel, *supra*, states that "[T]ranscripts for DDR1 are found in highly invasive tumor cells ... suggesting an involvement of DDR1 in tumor progression." See S78. Similarly, the abstract of Nemoto *et al.*, Pathobiology, vol. 65(4), pp. 195-203, 1997, relates that "the magnitude of mRNA expression [of DDR] was positively correlated with the proliferative activity of carcinoma cells" (see abstract appended to this paper). Vogel, *supra*, concludes that "cellular transformation such as invasion and metastasis might be mediated by DDRs," and that other human diseases that may involve "aberrant DDR expression or signaling" include "lung fibrosis, liver cirrhosis, osteoporosis, and rheumatoid arthritis." See, S81.

Similarly, it is well established that collagen is a MCK-10 signaling molecule, or ligand, which triggers a variety of subsequent actions, such as receptor dimerization and

transphosphorylation of the protein. Hence, Vogel *et al.*, *Mol. Cell.*, vol. 1(1), pp. 13-23, 1997, (abstract enclosed herewith) showed that "collagen activation of DDR1 induces phosphorylation of a docking site for the Shc phosphotyrosine binding domain" and concluded that DDR tyrosine kinases are "novel collagen receptors with the potential to control cellular responses to the extracellular matrix."

Eight years after the present application was filed, Vogel, 2001, remarked that it would be desirous to identify "suitable reagents such as monoclonal antibodies or dominant negative receptors that inhibit DDR signaling" (emphasis added), for the same reason Applicants' claimed invention seeks to identify antagonists and modulators of MCK-10 activity.

Accordingly, there is no question that MCK-10 is upregulated in cancerous cells, that there exist ligands that stimulate MCK-10 activity, or that there is a specific and substantial utility associated with the presently claimed invention. Therefore, Applicants respectfully request that Examiner withdraw this rejection.

II. The present claims are enabled

The Examiner rejected claims "75-87" under 35 U.S.C. § 112, first paragraph because, according to the Examiner, "even if the specification was enabling of how to use the polypeptide of SEQ ID NO: 2, the claims would not be enabled as written." Office Action at page 6.

The Examiner takes issue with claims 78, 81, and 85 because "a test compound could bind to the MCK-10 protein and prevent binding of the ligand, but the test compound could also activate the receptor and therefor [sic] act as an agonist." The Examiner also alleges that "there is ligand identified for the MCK-10 protein." Office Action at page 6. With respect to the latter point, Applicants have pointed out above that collagen is one, well-established, ligand for MCK-10.

Without acquiescing to the Examiner's point of view, Applicants have amended the claims to clarify that an *antagonistic* test compound of MCK-10 activity is one that inhibits or

reduces MCK-10 activity. Accordingly, the Examiner's speculative query is no longer applicable.

III. The present claims are not indefinite

The Examiner rejected claims 78-87 under 35 U.S.C. § 112, second paragraph because the independent claims "are incomplete method claims." Office Action at page 7. In elaborating, the Examiner contends that "in claims 78 and 85, it is not stated how to determine whether the test compound inhibits binding of the ligand to the MCK-10 protein. In claim 81, it is not clear what cellular change occurs in the cell line. In claim 82, there is no step of isolating the peptide. In claims 83 and 86, there are no steps that describe how to determine if the MCK-10 activity is modulated."

In response, Applicants contend that the positive methodological steps recited in the claims at issue are well within the understanding and purview of a person of ordinary skill in the art. He or she would know that it is possible to isolate protein complexes after various combinations of MCK-10, a test compound, and a natural ligand, such as collagen, are mixed together. Accordingly, the person of ordinary skill will be able to ascertain, quite easily, whether or not a test compound has displaced or prevented ligand binding.

Similarly, he or she would very easily be able to ascertain whether a "cellular change," relevant to MCK-10 activity, has occurred in cells that have been exposed to an antagonistic test compound. For example, the person of ordinary skill in the art would know that they could perform a phosphorylation profile, *i.e.*, a "phospho-blot," of the cell before and after exposure to the test compound. This is a routine laboratory procedure, which could reveal a difference in cellular phosphorylation profiles indicative of a change in cellular activity of the cell. However, any of a number of downstream signaling events could be profiled or measured. For instance, it is well known that ligand binding to receptor tyrosine kinases induces receptor dimerization, which further induces a variety of downstream events. Accordingly, the skilled artisan could ascertain whether exposure to the test compound prevents receptor dimerization, as an indicator of "cellular change."

As for claim 82, the Examiner states that there is no step of "isolating the peptide." However, subsection (b) of claim 82 requires "isolating a complex comprising an (i) MCK-10 protein, or splice variant thereof, *and (ii) a peptide*" (emphasis added). Subsection (c) entails determining the sequence of the peptide. The skilled artisan would recognize that an intermediate methodological step for "determining" the sequence of the peptide would entail isolating the peptide from the isolated complex and sequencing it. For the latter step, there a numerous vendors that provide protein sequencing services. Alternatively, protein sequencing may be carried out on automated machines or manually.

All of such exemplary and routine methods were well within the capabilities of the skilled person at the time the present invention was filed. The MANUAL OF PATENT EXAMINING PROCEDURE is clear in this regard. Section 2164.08 of the MPEP relates that, while the specification must teach those skilled in the art how to make and use the claimed invention without undue experimentation, "not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). The MPEP continues "all that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art."

Applicants have satisfied these examination guidelines and pointed out to the Examiner the various, routine methodological options that the skilled person has at their disposal for carrying out the presently claimed methods. Accordingly, Applicants respectfully request that the Examiner withdraw this rejection.

IV Conclusion

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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Discoidin domain receptors: structural relations and functional implications

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ABSTRACT **Multicellular life relies on the presence of extracellular matrix to provide scaffolding for cells and tissue compartments. To provide communication between cells and tissues, a multitude of cell surface receptors are triggered by soluble ligands and components of the extracellular matrix. A large family of these receptors transmit signals through the use of an intrinsic tyrosine kinase function. The subgroup of discoidin domain receptors (DDRs) is distinguished from other members of the receptor tyrosine kinase family by a discoidin homology repeat in their extracellular domains that is also found in a variety of other transmembrane and secreted proteins. Sequence comparisons show that non-mammalian orthologs of DDRs exist: three closely related genes in *Caenorhabditis* and one in the sponge *Geodia cydonium*. Recently, various types of collagen have been identified as the ligands for the two mammalian discoidin domain receptor tyrosine kinases, DDR1 and DDR2. The binding of collagen to DDRs results in a delayed but sustained tyrosine kinase activation. Both receptors display several potential tyrosine phosphorylation sites that are able to relay the signal by interacting with cytoplasmic effector proteins. The potential cross-talk to other receptors for collagen and the clinical aspects of DDR function are discussed.—Vogel, W. Discoidin domain receptors: structural relations and functional implications. *FASEB J.* 13 (Suppl.), S77–S82 (1999)**

Key Words: receptor tyrosine kinase · collagen signaling

DURING THE SEARCH for tyrosine kinase proteins expressed in human malignancies, a novel subfamily of receptor tyrosine kinases (RTK)[2] was discovered. This subfamily is distinct from other members of the large RTK group due to a homology domain to discoidin, a lectin first described during the cell aggregation process of the slime mold *Dictyostelium discoideum* (1, 2). All members share the approximately 160-amino-acid-long amino-terminal discoidin (DS) homology domain followed by a single transmembrane region, an extended juxtamembrane region, and a catalytic tyrosine kinase domain. The cDNAs of this novel RTK subfamily have been cloned from different species by many laboratories. They represent two distinct genes, which have now been renamed DDR1 [previously called DDR, NEP, Cak, Ptk-3, TrkE, MCK-10, RTK6, EDDR1, or NTRK4 (3–11)] and DDR2 [CCK-2, Tyro10, or TKT (8, 12, 13)]. The homology to the *Dictyostelium* discoidin protein resulted in the implication that DDRs function in cell adhesion, although nothing was known about their ligands at that time (5). The recent identification of collagen as a physiological ligand for these orphan receptors will allow further elucidation of the role of DDRs in cellular processes (14, 15).

DDR1 appears in three isoforms a, b, and c, which are generated by alternative splicing. The exon 11, coding for an additional 37 amino acids in the juxtamembrane region, is missing in the transcript of DDR1a, but is present in DDR1b (8, 16, 17). Another six amino acids (S-F-S-L-F-S) are inserted just at the beginning of the kinase domain to give rise to the c-isoform. Recently, a similar sequence (S-L-S-V-A-Q) was found to be alternatively spliced in the cytoplasmic domain β_1 integrin (18). During rat postnatal development, the amount of DDR1b considerably increases in comparison to the a-isoform (19). DDR1 and DDR2 are detected as approximately 125- and 130-kDa glycosylated proteins in a Western blot of lysates from overexpressing cells (8). Treatment with tunicamycin shows DDR1a and DDR1b as 102- and 106-kDa proteins, respectively, thereby suggesting that the shorter isoform is more heavily glycosylated than the longer one (unpublished observation). DDR1 is partially processed into a 63-kDa membrane-anchored b-subunit and a soluble 54-kDa a-subunit by a so far unidentified protease (8). The presence of the recognition sequence RXRR in the extracellular domain suggests the involvement of furin-like proteases in DDR1 processing.

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[2] Abbreviations: DDR, discoidin domain receptor; RTK, receptor tryosine kinases; DS, discoidin; PTB, phosphotyrosine binding.

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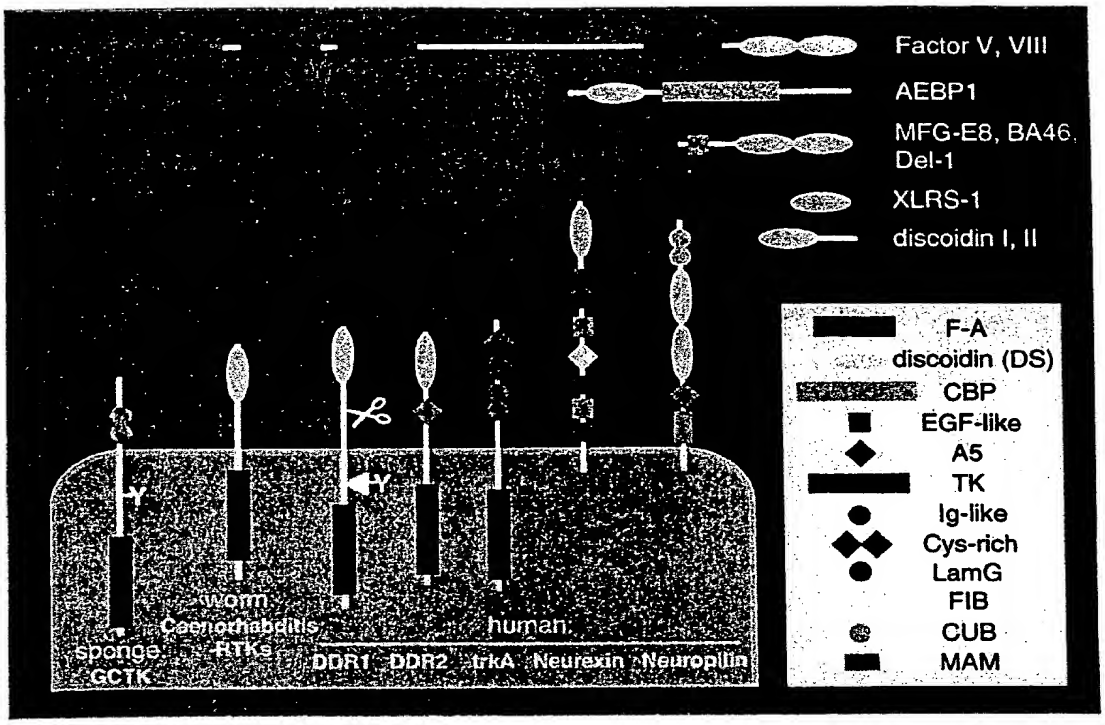

Figure 1. The family of proteins with DS domains. Schematic representation of transmembrane and secreted proteins. Abbreviations for the domains shown are as follows: F-A, A-domain in Factor V and VIII; CBP, carboxypeptidase; EGF, epidermal growth factor; TK, tyrosine kinase; A5, homology to A5 antigen; Ig, immunoglobulin; LamG, laminin-G; FIB, fibronectin-like; CUB, complement binding; and MAM, meprin/A5/PTPmu. The tyrosines in the N-P-X-Y motives of GCTK and DDR1b are highlighted. Proteolytic processing of DDR1 is indicated.

EXPRESSION PATTERNS OF DDRS

Using Northern blot and *in situ* hybridization, both DDRs have been found widely expressed in human and mouse tissues. DDR1 is abundant in the brain and is found in keratinocytes, in the epithelial layer of the colonic mucosa, in the distal tubulus of the kidney, in the lung epithelium, and in the thyroid follicles (3, 5–9). In the pancreas, DDR1 expression is restricted to the islets of Langerhans (8). During mouse development, DDR1 can be used as an early marker for the formation of neuroectodermal cells (4). In contrast, little is known about the occurrence of DDR2. Northern blot analysis indicates expression of DDR2 in heart and skeletal muscle, lung, brain, kidney, and connective tissue (8, 12, 13).

Five different studies have detected an overexpression of DDR1 in human tumors, particularly in primary breast cancer (20), but also in ovarian (9), esophageal (21), and up to threefold in pediatric brain cancer (22). More importantly, *in situ* hybridization of adjacent sections of ovarian and lung tumors revealed a mutually exclusive expression of DDR1 and DDR2 (8). Transcripts for DDR1 are found in highly invasive tumor cells, whereas transcripts for DDR2 are detected only in the surrounding stromal cells, suggesting an involvement of DDR1 and DDR2 in tumor progression. The expression of DDR1 can either be induced by γ-radiation of rat astrocytes (19) or by the overexpression of p53 in osteosarcoma cells (23). A targeted deletion of DDR1 or DDR2 in embryonic stem cells will allow the analysis of cellular signaling in the absence of either of the DDRs. The generation of knock-out mice will address the role of DDRs during embryogenesis and later development.

DDR-RELATED PROTEINS

Database searches have identified three sequences with homology to DDR1 and DDR2 in the genomes of nematodes, two in *Caenorhabditis elegans*, and one in *Caenorhabditis briggsae* (24). All three proteins show the common features of DDRs: an amino-terminal DS domain and a very long juxtamembrane stretch followed by the catalytic tyrosine kinase domain (**Fig. 1**). Because these genes were identified during the worm genome project, nothing is known about their functional significance. Nevertheless, these receptors could well be activated by collagen. It has been recently shown that related RTKs are able

to execute similar functions in nematodes and in mammals, for example the Eph-receptors in the developing nervous system (25).

It is surprising to note that the closest relative to the tyrosine kinase domain of DDR1 is found in the genome of the marine sponge *Geodia cydonium* (Fig. 1). The multiple sequence alignment shows that the catalytic core region of DDR1 is 59% identical to the *Geodia* tyrosine kinase called GCTK (61% for DDR2), whereas the closest mammalian RTK subfamily, the neurotrophin receptors, are only 55–58% related (26). A common ancestor for the *Geodia* and human protein would therefore date back more than 600 million years, during the early evolution of multicellular organisms. Detailed sequence analysis revealed that several tyrosines in the cytoplasmic domain are conserved and may function as autophosphorylation sites. The juxtamembrane region of the sponge protein includes the sequence N-P-X-Y, a sequence also seen in the alternatively spliced exon of DDR1 (8, 26). A signaling pathway similar to that in mammals can be postulated in sponges if GCTK activation leads to phosphorylation of the N-P-X-Y site. It is interesting to note that the sponge RTK lacks the amino-terminal DS-homology region found in the DDRs and instead shows two immunoglobulin (Ig) folds (26). A similar Ig-domain repeat is found in the mammalian nerve growth factor receptors, which are the second-most-related RTK subfamily to the *Geodia* sequence (Fig. 1). Previous results suggest that sponges are utilizing their collagen matrix to maintain a polarized epithelial layer (27). One may speculate that sponges employ GCTK in cell-to-extracellular matrix interactions, possibly for the differentiation of sensory cells.

Two other mammalian, non-tyrosine kinase receptors show DS domains: the neuropilins and the neurexins. As their names imply, both are involved in the development of the nervous system. The neuropilins, and their much earlier identified *Xenopus laevis* counterpart called A5 antigen, are receptors for the semaphorins, a family of secreted and transmembrane glycoproteins, and for certain isoforms of vascular endothelial growth factor (28). Neuropilins have a tandem repeat of DS domains flanked by several other domains that are commonly found in cell adhesion proteins. It is interesting to note that the approximately 80-amino-acid stretch following the DS domains in neuropilin/A5 antigen has sequence homology to the corresponding region in the extracellular domain of DDR2 (amino acids 254–336) (12). During sensory and motor neuronal patterning, the interaction between neuropilins and semaphorins results in growth cone collapse (28). The neurexins appear to mediate cell-cell contacts, forming a trimeric complex between neurexin, contactin, and RPTPβ at the junction of neurons with glial cells (29).

DS domains are found in a variety of secreted proteins, most notably as a tandem carboxy-terminal repeat in the blood coagulation cofactors V and VIII (Fig. 1). During blood coagulation, the DS domains of factor V and VIII are thought to bind to phospholipids at the surface of platelets (30). The three-dimensional structure of the DS domains of factor V has been recently predicted using the homologous structure of galactose oxidase (31). In an adipocyte transcriptional repressor protein, called AEBP1, the DS domain is followed by a domain with carboxypeptidase activity (32). The MFG-E8, BA46, Del-1 group of DS-domain proteins have a similar overall structure but diverse expression and function: MFG-E8 was originally identified as milk fat protein, but was also found at the acrosomal cap of sperm cells, suggesting a role in fertilization (33, 34); BA46 (also called lactadherin) was found to be expressed in breast carcinomas as well as in human milk fat (35–37), whereas Del-1 is expressed in endothelial cells (38). Both proteins are suggested to be involved in $\alpha_v\beta_3$ integrin-mediated cell adhesion (36, 38). A human X-linked inherited disease (retinoschisis) resulting in retinal deterioration was mapped to the XLRS-1 gene (39). XLRS-1 codes for a 224-amino-acid protein, which almost entirely consists of a single DS domain. A majority of the retinoschisis patients show missense mutations in the DS domain sequence affecting conserved amino acid residues (40). So far little is known about the function of XLRS-1 in early eye development. The progenitors of all DS domains, the discoidin I and II proteins, are produced during cell aggregation of *Dictyostelium* and are secreted while the amoebas convert into a multicellular organism (1, 2). Discoidins are lectins binding to galactose and *N*-acetyl-galactosamine. It is presently unknown whether any of the mammalian proteins with DS domains could act as lectins as well.

COLLAGEN AS SIGNALING MOLECULE

The revelation that collagens function as ligands for DDR1 and DDR2 suggests that some of the other mammalian DS domains may interact with matrix proteins as well. To date, 19 different collagens have been described. Collagen type I, II, III, and IV are ubiquitous and tend to form fibers or sheet-like structures. The others appear to regulate the size of fibers or have more restricted expression pattern in specialized cells and tissues (41). Point mutations or deletions in genes coding for collagens can impair collagen assembly and result in severe disorders of the skeleton, tendon, or skin.

DDR1 is activated by all collagens so far tested

(type I to type V), whereas DDR2 is selectively stimulated by fibrillar collagens only (14, 15). The activation process is surprisingly slow, requiring collagen treatment for 18 h to reach maximal tyrosine kinase activity. The activation is sustained and no significant down-regulation by endocytosis or receptor degradation is observed for up to 4 days. The native, triplet configuration of collagen is essential for DDR1 and DDR2 activation, whereas collagen glycosylation is only important for DDR2 stimulation (14). It is not yet clear which part of the DDR extracellular domain is essential for ligand binding. Conversely, the regions in the collagen molecule that harbor the binding epitopes for DDR1 or DDR2 are not yet defined. A synthetic collagen, comprised of 10 collagen repeats [$(Gly\text{-}Pro\text{-}Hyp)_{10}$] and cross-linked to form a mini-triple helix, is not sufficient to activate DDRs (unpublished observation). The functional significance of DDR signaling waits to be fully determined. However, it was found in an initial experiment that prolonged activation of DDR2 by collagen is associated with an up-regulation of matrix-metalloprotease-1 (MMP1), an enzyme that specifically cleaves native fibrillar collagen (14). The culture of human skin fibroblasts in three-dimensional collagen gels induces down-regulation of collagen synthesis and a tyrosine kinase-dependent up-regulation of MMP1 expression (42). Mechanical force applied to cells may influence DDR signal transduction as well, as suggested by the recent observation that MMP1 is up-regulated in response to stretch relaxation of fibroblasts (43).

Ligand binding to RTKs is thought to induce receptor dimerization and subsequent transphosphorylation of specific tyrosine residues in the cytoplasmic domain. These tyrosines are embedded in consensus sequences, which on phosphorylation allow the binding of downstream effector molecules containing Src-homology 2 (SH2) or phosphotyrosine-binding (PTB) domains (44). The activation of DDR1 by collagen induces phosphorylation of the L-X-N-P-X-Y site in the alternatively spliced insert. The adaptor molecule Shc, which consists of an amino-terminal PTB and a carbxy-terminal SH2 domain, binds with its PTB domain to this site (14). Another PTB-domain-containing protein, FRS2, has been shown to interact with the juxtamembrane region of the a-isoform of DDR1 (Foehr, E. D., Tatavos, A., Tanabe, E., Raffioni, S., Goetz, S., DiMarco, E., DeLuca, M., and Bradshaw, R. A., unpublished observations). However, it remains to be shown that the DDR1a-FRS2 interaction requires ligand-induced receptor activation. Other tyrosines that are conserved in DDR1 and DDR2 display the consensus sequence for the SH2 domains of Nck, GAP, and the p85 subunit of PI3-kinase. A potential

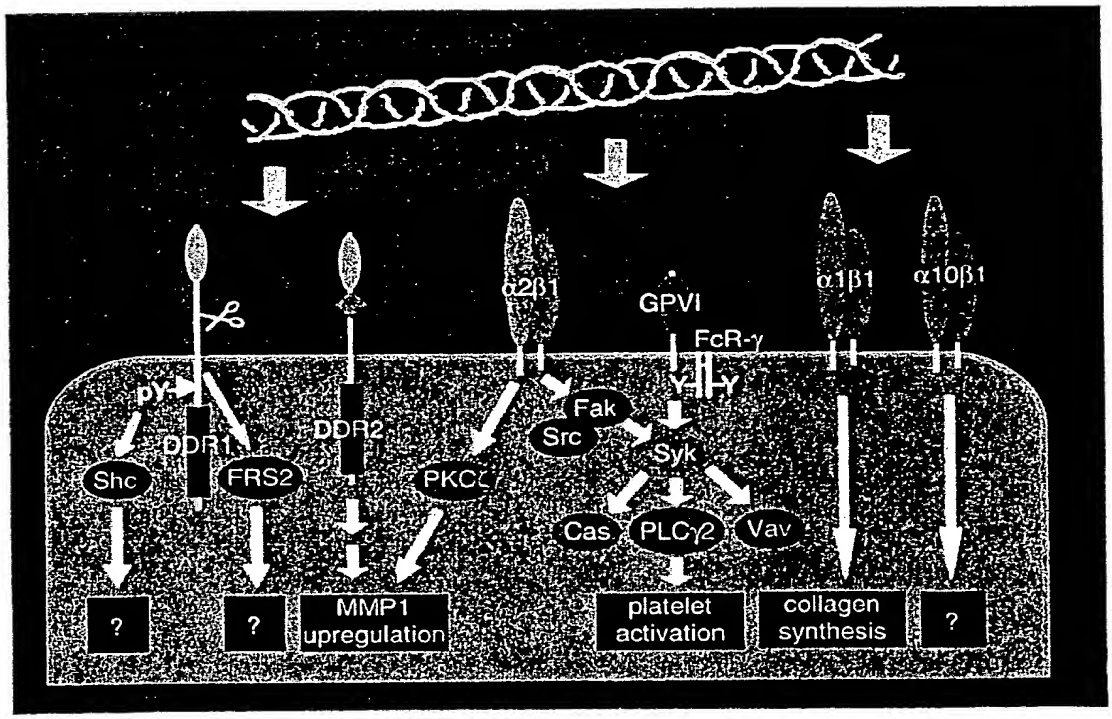

Figure 2. Receptors for collagen and their potential downstream signaling pathways. It is worth noting that these receptors are expressed in a variety of different cell types. The depicted pathways and cellular responses are most likely more complex because they are also triggered by other extracellular stimuli than collagen. Abbreviations are described in the text.

binding site for proteins with SH3 domains (P-R-G-P-G-P-P-T-P) is present in the alternatively spliced exon of DDR1. Furthermore, the sequence L-N-T-V at the carboxy terminus of DDR1 may serve as binding site for proteins with PDZ domains.

Collagen acts not only as a ligand for DDRs, but binds and activates members of the integrin family, which are heterodimers of one α and one β subunit (**Fig. 2**). The $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_{10}\beta_1$ integrins have been shown to be functional receptors for collagen (45–47). Lacking intrinsic catalytic activity, integrins mediate their responses using cytoplasmic tyrosine kinases, particularly Fak, Syk, and Src. The interaction of integrins with collagen has been extensively studied in various cell types, including fibroblasts and platelets. In platelets, the contact of $\alpha_2\beta_1$ with collagen and $\alpha_{IIb}\beta_3$ with fibrinogen leads to an increase in tyrosine phosphorylation of phospholipase Cγ2 (PLCγ2), Vav, and Cas, an adaptor molecule, that provides multiple SH2 domain interaction sites (48). The complex between the Fc receptor γ-chain and the uncharacterized glycoprotein VI (GPVI) serves as additional collagen receptor in platelets (Fig. 2). In fibroblasts, the formation of the cytoskeleton is regulated mainly by the action of integrins, and an involvement of the signal of DDRs in maintaining the filamentous network could be envisioned. Experiments in three-dimensional collagen gels will address the question of whether DDR phosphorylation can be induced by mechanical forces. Initial data from epithelial cells and fibroblasts indicate that DDR1 signaling takes place independent of β_1 integrin activation.

CLINICAL PROSPECTS

The overexpression of DDR1 in several different human cancers suggests a function in tumor progression. Although experimental evidence argues against a classification of DDRs as transforming oncogenes, subsequent steps after the initial cellular transformation such as invasion and metastasis might be mediated by DDRs. The ability of malignant cells to penetrate tissue barriers requires the breakdown of extracellular matrix, which is mediated by a variety of proteases (49). The observed up-regulation of MMP1 by collagen-activated DDR2 is potentially only one out of several downstream targets. Other human diseases with deregulated matrix production, including lung fibrosis, liver cirrhosis, osteoporosis, and rheumatoid arthritis may present aberrant DDR expression or signaling.

It is tempting to speculate that one major function of DDRs is to monitor the formation of collagenous extracellular matrices by regulating the synthesis of collagens and their degrading enzymes. The challenge in future work will be to identify the relevant receptor targets and to analyze DDR function by designing suitable reagents such as monoclonal antibodies or dominant negative receptors that inhibit DDR signaling.

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Overexpression of protein tyrosine kinases in human esophageal cancer.

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Using a PCR-based cloning technique, we isolated a series of protein tyrosine kinases (PTKs) expressed in a cell line of esophageal squamous cell carcinoma. Sequence analysis revealed 10 different kinds of PTKs of the receptor type [epidermal cell growth factor receptor, insulin-like growth factor I receptor, fibroblast growth factor receptor 4, eck, erk discoidin domain receptor (DDR)/trkE/cell adhesion kinase (Cak), HEK2, HEK8, axl and sky] and one PTK of the nonreceptor type (tyk2). Subsequently, we examined the expression of the transcripts of these 11 genes in paired samples of normal and carcinomatous esophageal tissues obtained from 12 cases of esophageal cancer. We found that 11 gene transcripts were expressed in both carcinomatous ar normal tissues, and 6 of them were significantly overexpress in carcinomatous tissues relative to adjacent normal tissues. Among these, the magnitude of mRNA expression of DDR/trkE/Cak PTK was positively correlated with the proliferative activity of carcinoma cells, but not with their degree of differentiation. Immunohistochemically, DDR was expressed in both normal and cancerous esophageal cells. Tr intensity of the expression was higher in cancer than normal tissue. In addition, we confirmed the expression of two isoforms of DDR/trkE/Cak in normal and cancerous esophagu Our study suggests that DDR/trkE/Cak plays an important ro in the regulation of proliferation of esophageal cancer.

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The discoidin domain receptor tyrosine kinases are activated by collagen.

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Two mammalian receptor tyrosine kinases (DDR1 and DDR2) have extracellular domains closely related to a D. discoideum lectin, discoidin, required for cell aggregation. Here, we show that the mammalian DDR receptors bind and are activated by specific types of collagen. Stimulation of DDR receptor tyrosine kinase activity requires the native triple-helical structure of collagen and occurs over an extended period of time. Collagen activation of DDR1 induces phosphorylation of a docking site for the Shc phosphotyrosine binding domain, whose presence is controlled by alternative splicing. Activation of DDR2 by collagen results in the up-regulation of matrix metalloproteinase-1 expression. These results suggest that the discoidin-related DDR tyrosine kinases are novel collagen receptors with the potential to control cellular responses to the extracellular matrix.

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